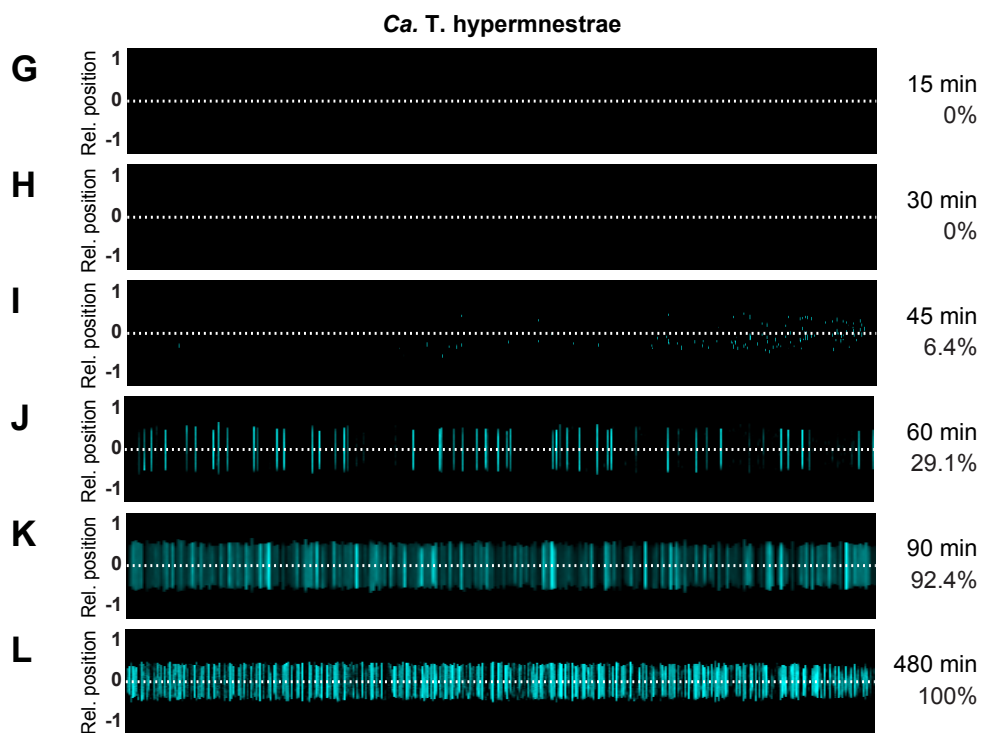
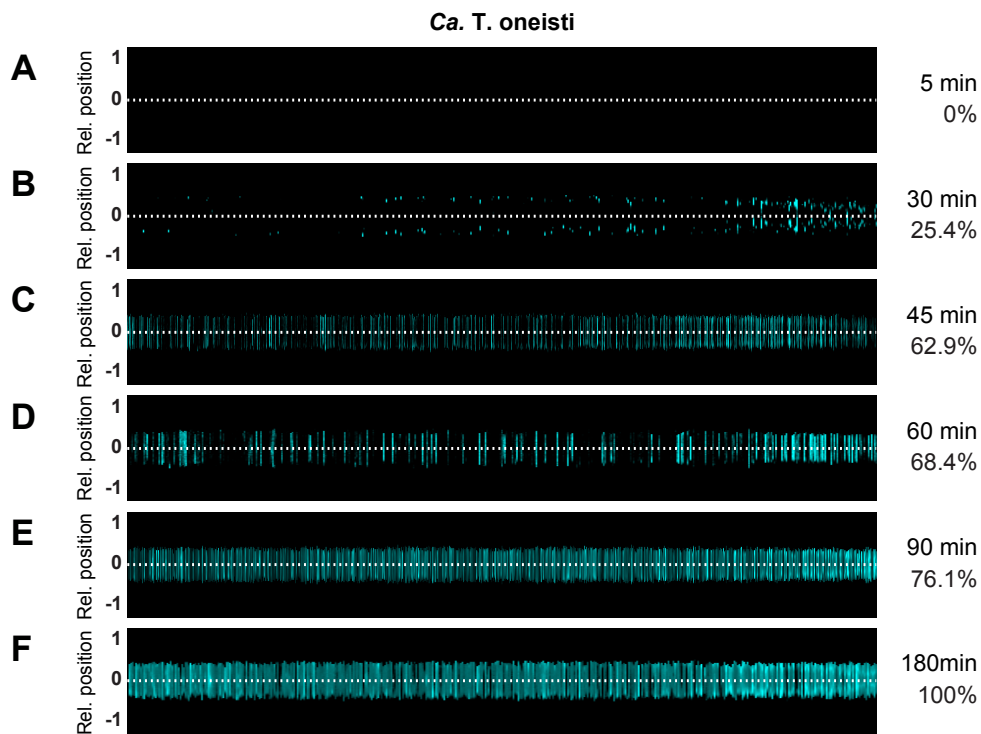


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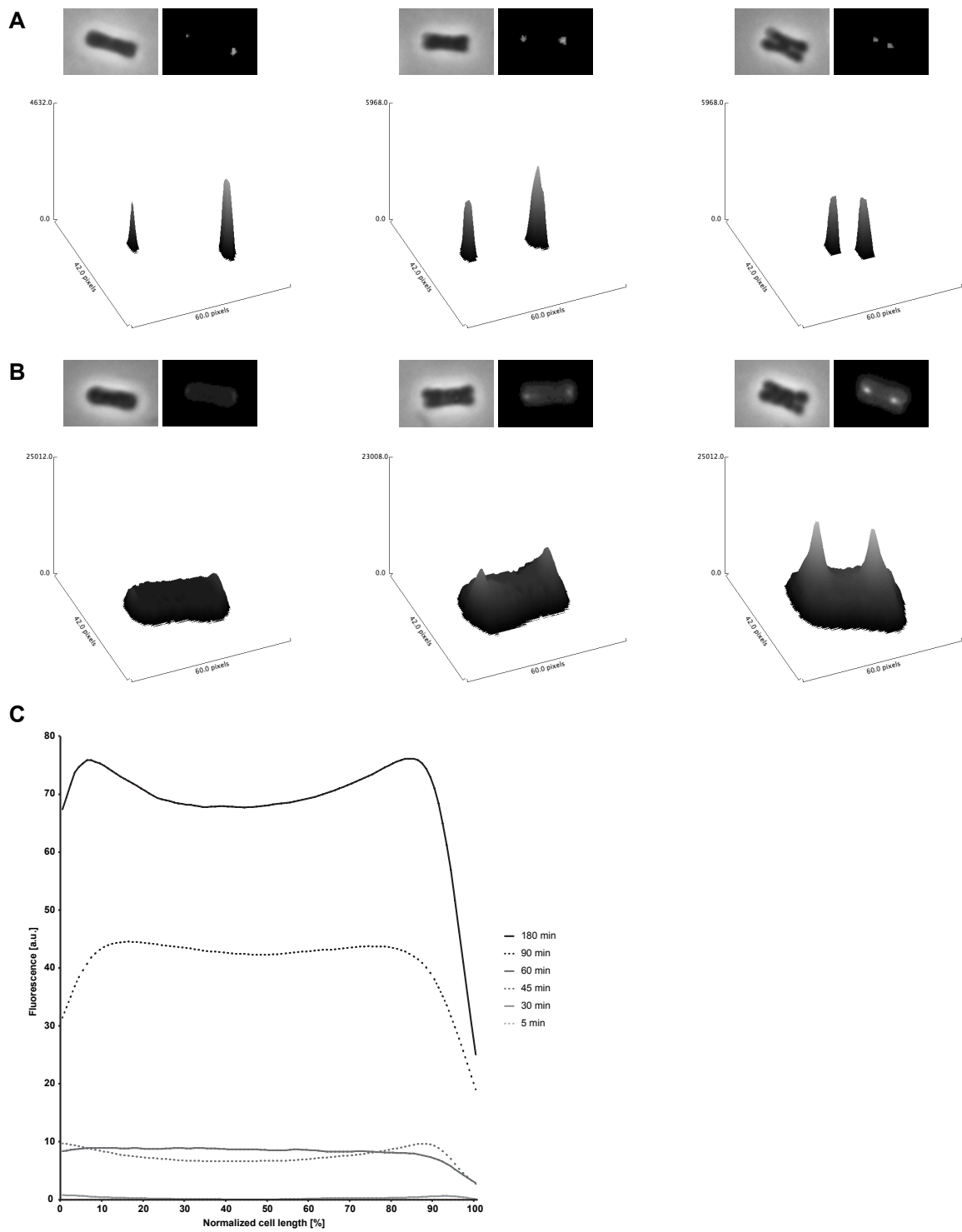
## **Supplemental Information**

### **Host-Polarized Cell Growth in Animal Symbionts**

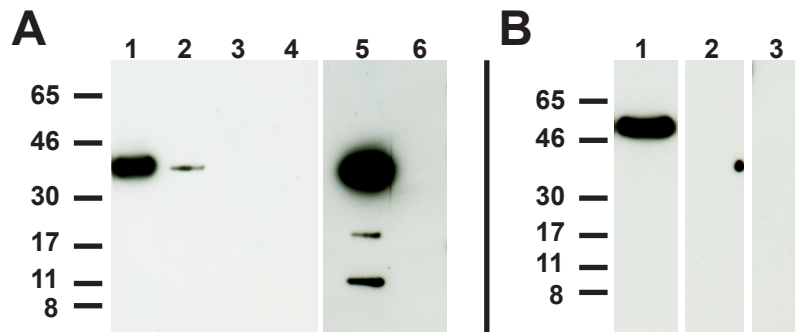
**Nika Pende, Jinglan Wang, Philipp M. Weber, Jolanda Verheul, Erkin Kuru, Simon K.-M.R. Rittmann, Nikolaus Leisch, Michael S. VanNieuwenhze, Yves V. Brun, Tanneke den Blaauwen, and Silvia Bulgheresi**



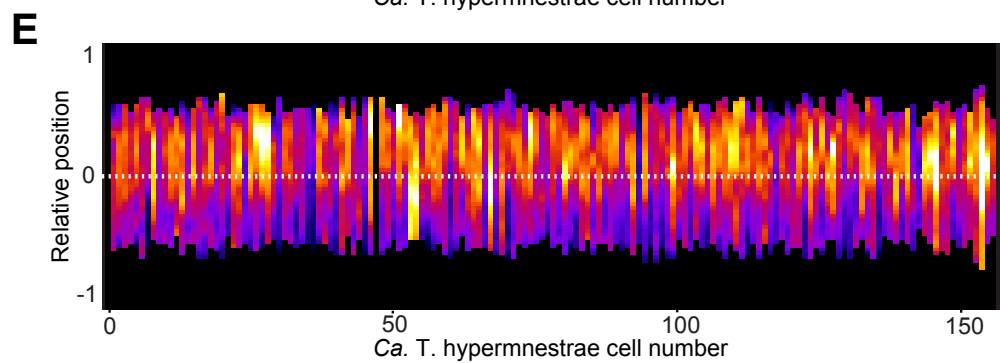
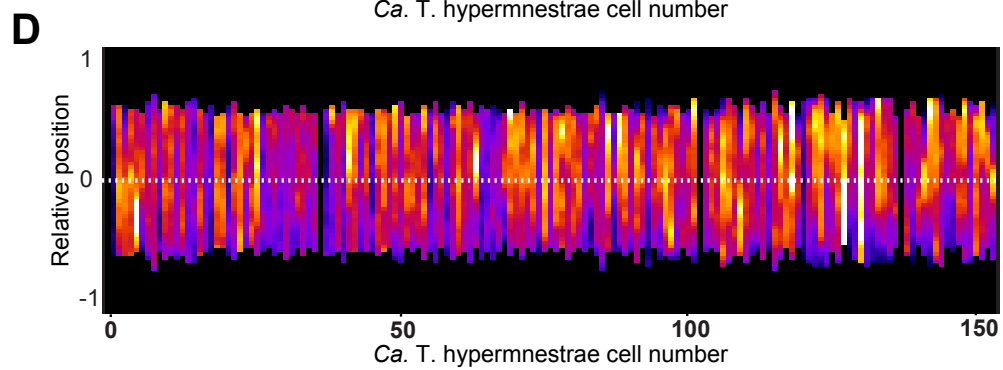
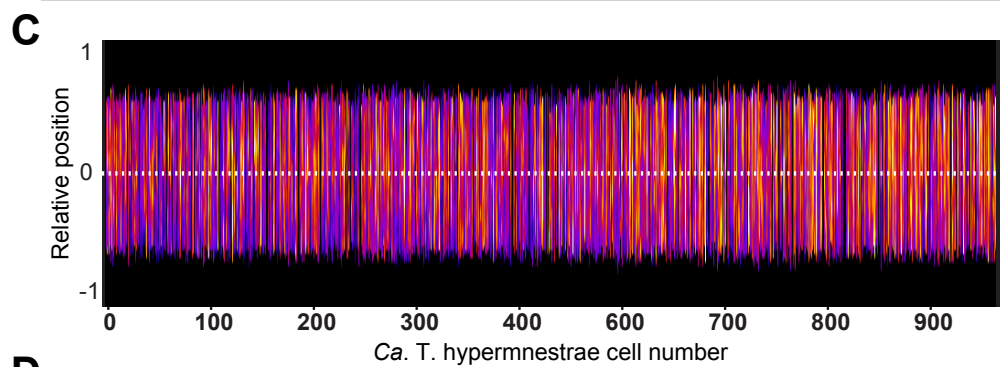
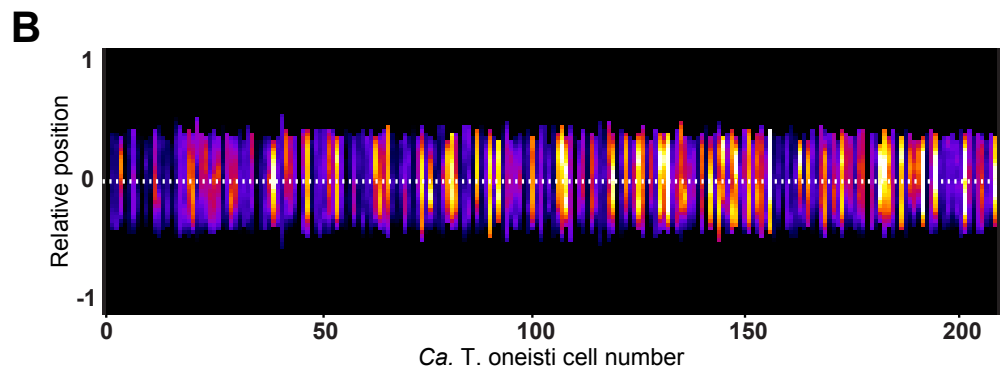
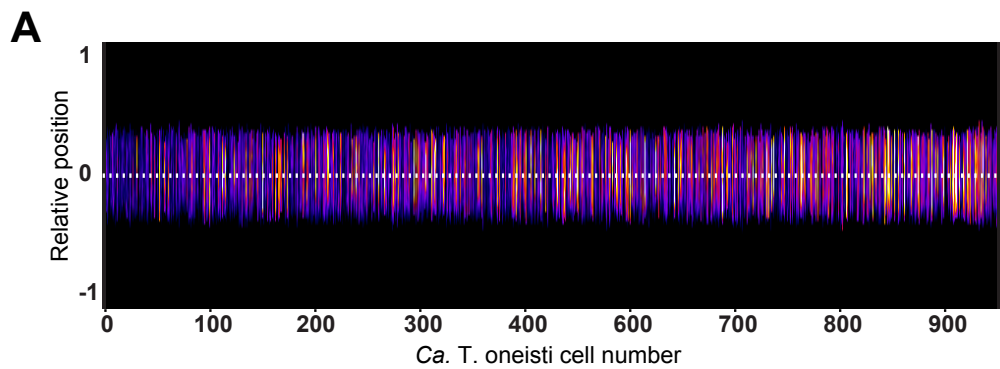
**Figure S1. Localization of newly synthesized peptidoglycan (PG) in *Ca. T. oneisti* and *T. hypermnestrae*, Related to Figure 1.** Demographs of *Ca. T. oneisti* (A-F) and *T. hypermnestrae* (G-L) incubated with EDA-DA. Each cell is represented as a pixel-wide bar whose length corresponds to the long axis of the cell. In the case of *Ca. T. hypermnestrae*, each bar is oriented such that the upper extremity corresponds to the distal cell pole and the lower extremity corresponds to the proximal pole. Symbiont cells were sorted according to increasing width from left to right. Dotted white line indicates the center of the cell long axis. Incubation time in minutes and % of EDA-DA labelled cells are written on the right side of each demograph. Each demograph was constructed with cells derived from three nematodes in the case of *Ca. T. oneisti*, and from a single nematode in the case of *Ca. T. hypermnestrae*. Total numbers of imaged *Ca. T. oneisti* cells per demograph were 383 (A), 567 (B), 1,762 (C), 500 (D), 798 (E), 454 (F). Total numbers of imaged *Ca. T. hypermnestrae* cells per demograph were 335 (G), 1,055 (H), 1,360 (I), 423 (J), 264 (K), 424 (L).



**Figure S2. Localization of newly synthesized peptidoglycan (PG) in *Ca. T. oneisti* incubated in EDA-DA, Related to Figure 1.** (A) Phase contrast images (left) and corresponding fluorescence images of EDA-DA signal (right) of the *Ca. T. oneisti* cells shown in Figure 1A and (B) phase contrast images (left) and corresponding fluorescence images of EDA-DA signal (right) of the *Ca. T. oneisti* cells shown in Figure 1C. In all the corresponding surface plots (bottom panels of A and B), x- and z- axis are the picture size in pixel and y-axis are the grey values. For both experiments, imaging exposure time was the same and no post-acquisition modification was performed. (C) EDA-DA fluorescence (a.u.) emitted from all the *Ca. T. oneisti* cells for each time point was averaged and plotted against normalized cell length (%). Total number of cells per time point were 383 (5min), 567 (30min), 1762 (45 min), 500 (60 min), 798 (90 min) and 454 (180 min).

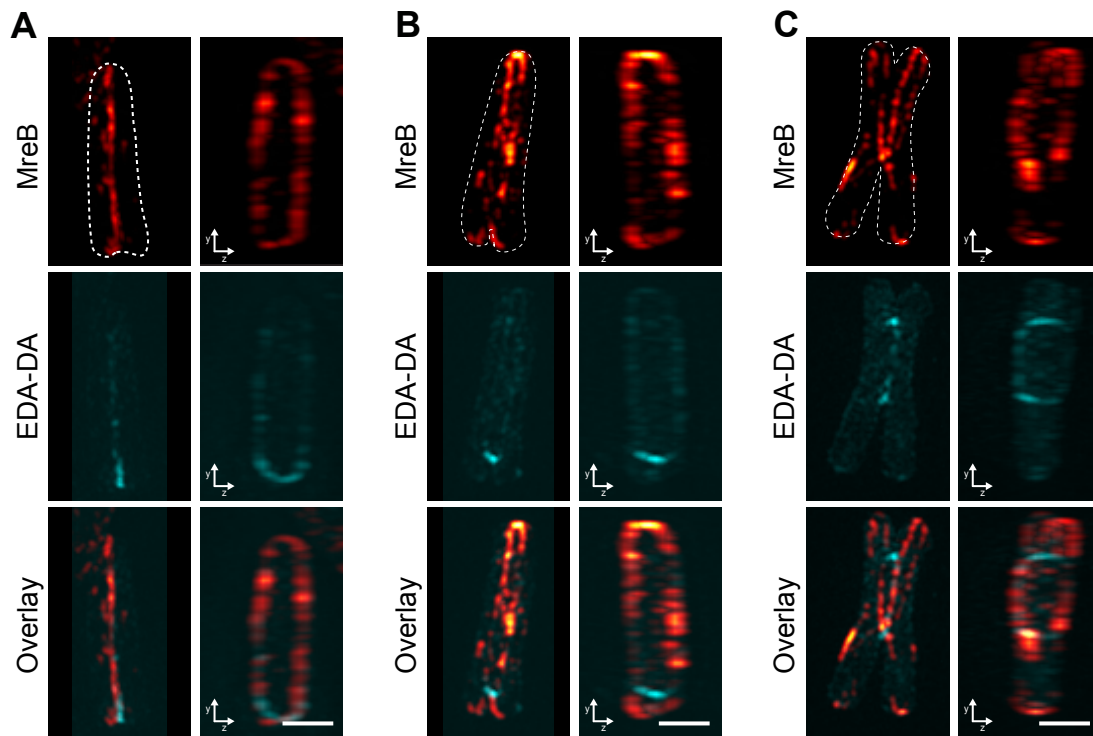


**Figure S3. Western blots of *Ca. T. oneisti* and *T. hypermnestrae* protein extracts, Related to Figures 3-6 and Figures S3-S6.** (A) Western blots of *Ca. T. oneisti* (lanes 1 and 3) and *Ca. T. hypermnestrae* (lane 2 and 4) protein extracts probed with immunoaffinity purified rabbit polyclonal anti-*E. coli* MreB antibody (lanes 1 and 2) or with the secondary antibody only (lanes 3 and 4). (B) Western blots of *Ca. T. oneisti* protein extracts probed with a rabbit polyclonal anti-*Ca. T. oneisti* FtsZ peptide antibody (lane 1), with the pre-immune serum (PI, lane 2) and with the secondary antibody only (lane 3). *Ca. T. oneisti* MreB predicted MW is 37 kDa and *Ca. T. hypermnestrae* MreB predicted MW is 38 kDa. Numbers indicate apparent MW expressed in kDa.

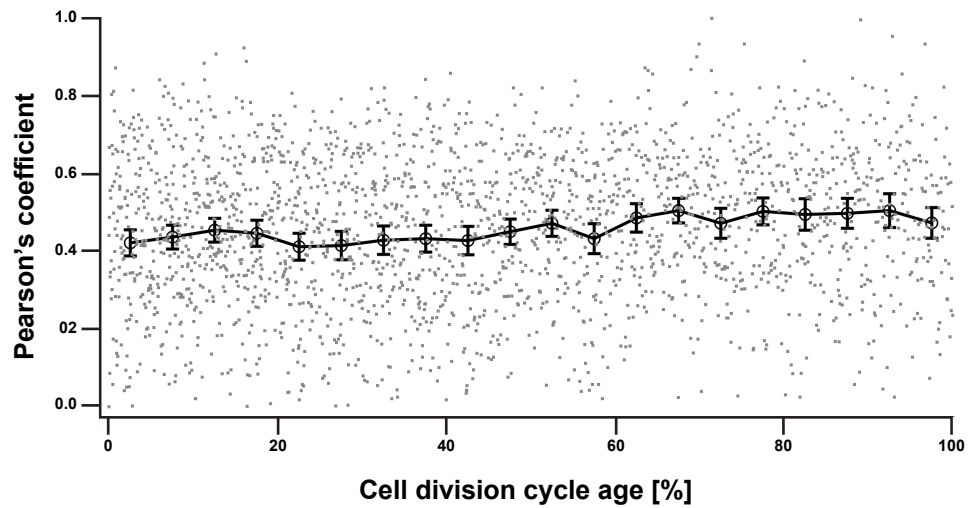


**Figure S4. MreB localization pattern in *Ca. T. oneisti* and *T. hypermnestrae*, Related to Figures 3 and 5.** (A-E) Demographs of *Ca. T. oneisti* (A-B) and *Ca. T. hypermnestrae* (C-E) immunostained with anti-MreB antibody. The MreB fluorescence emitted by each cell is represented as a pixel-wide bar whose length corresponds to the long axis of the cell. Symbiont cells were arranged according to increasing width from left to right. In the case of *Ca. T. hypermnestrae* (C-E), the bar is oriented such that the extremity corresponding to the proximal cell pole is down and the extremity corresponding to the distal cell pole is up. Dotted white line indicates the center of the cell long axis. *Ca. T. oneisti* cells were divided into two morphological classes, non-constricted cells (A; n=924) and constricted cells (B; n=209). *Ca. T. hypermnestrae* cells were divided into three morphological classes: non-constricted cells (C; n=973), proximally constricted cells (D; n=154) and proximally and distally constricted cells (E; n=155).





**Figure S5. 3D SIM analysis of MreB localization pattern in *Ca. T. hypermnestrae* incubated with the PG metabolic probe EDA-DA, Related to Figures 1 and 4.** (A-C) Representative proximally indented (A), proximally constricted (B) and proximally and distally constricted (C) *Ca. T. hypermnestrae* cells incubated with the clickable PG precursor EDA-DA and immunostained with anti-*E. coli* MreB antibody. Dotted white line indicates cell outline. Front view (left) and a corresponding 90° shifted side view (right) is shown of each cell. Scale bar is 1  $\mu\text{m}$ .



**Figure S6. Pearson's coefficient for MreB and FtsZ fluorescence, Related to Figure 5.**

The Pearson's coefficient was determined for each of the 2,308 cells immunostained with anti-MreB and anti-FtsZ antibodies (dots in the graph). Subsequently, the data were binned in age classes of 5%. The plotted line connects the averages of the bins, and the error bars show the 95% confidence per bin. Cell age (expressed as cell division cycle age %) is calculated from its contour area with respect to the entire population which is assumed to grow exponentially.

Species	Sequence	Position
EcMreB	-MLKKFRGMFSNDLSIDLGTANTLIYVKGGQIVLNEPSVVAIRQDRAGSPKSVAAVGHDA	59
ToMreB	MFLRRIRGIFSNDSIDLGTANTLIYTRGGQIVLNEPSVVAIRQ-GPGGSKSVAAVGNEA	59
ThMreB	MFLRRIRGVFSNDLSIDLGTANTLIYIRGGQIVLNEPSVVAIRQGGGGPKSVAAVGTEA	60
	:*::*:*****::*****:***** * . ***** :*	
EcMreB	KQMLGRTPGNIAAIRPMKDGVIADFFVTEKMLQHFQVHSNSFMRPSRVLVCPVPGAT	119
ToMreB	KQMLGRTPQNITAIRPLKDGVIADFTVTEKMLQFFIHKVHESRIIRPSRVLICVPCGST	119
ThMreB	KQMLGRTPKNIMAVRPLMDGVIADFTVTEKMLQYFIHKVHESRLIRPSRVLICVPCGST	120
	***** * *:*: ***** *****.*::*.. :*****:*** *:*	
EcMreB	QVERRAIRESAQGAGAREVFLIEPMAAAIGAGLPVSEATGSMVVDIGGGTTEVAVISLN	179
ToMreB	QVERRAIKESAAGAGAREVFLIEPMAAAIGAGLPVDEARGSMVLDVGGGTSEVAITSLN	179
ThMreB	QVERRAIKESAAGAGAREVFLIEPMAAAIGAGLPVEEARGSMVLDIGGGTSEVAILSLN	180
	*****:*** **********.* *****:***:****: ***	
EcMreB	GVVYSSSVRIGGDRDFDEAIINYVRRNYGSLIGEATAERIKHEIGSAYPG--DEVREIEVR	237
ToMreB	GIVYSNSVRIGGDRFDDAITAYVRRNYGALIGEVTAEHIKYEIGSAYPG--GEMREIEVM	237
ThMreB	GIVYSNSVRIGGDRFNEAIYVRRRHGALIGEATAEYIKHKIGSAYPDSGNEVREVEFR	240
	*:***.*****:*** *****.*::*****. .*:***.	
EcMreB	GRNLAEGVPRGFTLNSNEILEALQEPLTGIVSAVMVALEQCPPELASDISERGMVLTTGGG	297
ToMreB	GRNLAEGVPRSFTLNSNEILEALQEPLSGIIEAVKTALEQIPPELGADVAERGIVTTGGG	297
ThMreB	GRYMAEGVPRSFTLNSNDVLEALQEPLAGICGTVKIALEQTPPELAADVAERGIVTTGGG	300
	** :*****.*****:*****:* :* *** *****.:*:*****:*****	
EcMreB	ALLRNLDRLMEETGIPVVVAEDPLTCVARGGGKALEMIDMHGG-DLFSEE-	347
ToMreB	ALLADIDRLLEEETGLPVLIADDPLTCVARGGGHALETTNER-SLDLLAMTE	348
ThMreB	ALLAHIDRLLEEETGLSVVVDADPLTCVARGGGRALEMVDKPSGLDLFTVVE	352
	*** :***** *****:*****:*****:*** :. .*::	

**Figure S7. Peptide sequence alignment of *E. coli* MreB (EcMreB; *E. coli* K12 MreB; AKK14940.2), *Ca. T. oneisti* MreB (ToMreB; MF350658) and *Ca. T. hypermnestreae* MreB (ThMreB; MF317948), Related to Figures 3-6.** Conserved amino acids involved in ATP binding site are highlighted in blue; amino acids at mutation sites that confer resistance to A22 [S1] are all conserved (grey) but one which is semi-conserved (Ser instead of Asn at position 185, yellow). The amino acid required for FtsZ-interaction [S2] is highlighted in turquoise. ToMreB and ThMreB are 77% and 76% identical and 87% and 88% similar to EcMreB, respectively.

Group and treatment	A22-treated I	A22-treated II	untreated I	untreated II
Initial # of worms	55	77	70	42
Time after treatment	Survival rate			
2h	100%	100%	100%	100%
4h	100%	100%	100%	100%
8h	100%	100%	100%	100%
24h	96.3%	100%	98.5%	100%
48h	96.3%	100%	98.5%	100%
72h	96.3%	98.7%	98.5%	100%

**Table S1. Survival rate of A22-treated and untreated *Caenorhabditis elegans*, Related to STAR Methods.**

Species	Treatment	n	Length Mean ( $\mu\text{m}$ )	Length StD ( $\mu\text{m}$ )	Width Mean ( $\mu\text{m}$ )	Width StD ( $\mu\text{m}$ )	Total
Ca. T.one	Control	1407	2.82	0.32	0.82	0.18	<b>n = 6793</b>  <b>Mean Length = 2.78 <math>\mu\text{m}</math></b>  <b>Mean Length StD = 0.30 <math>\mu\text{m}</math></b>  <b>Mean Width = 0.87 <math>\mu\text{m}</math></b>  <b>Mean Width StD = 0.18 <math>\mu\text{m}</math></b>
		739	2.79	0.19	0.87	0.18	
		906	2.95	0.16	0.91	0.20	
		603	2.34	0.20	0.89	0.19	
		289	2.72	0.20	0.86	0.19	
		327	2.73	0.26	0.90	0.19	
		573	2.62	0.28	0.82	0.14	
		693	2.87	0.21	0.87	0.16	
		425	2.83	0.24	0.88	0.15	
		831	2.89	0.28	0.88	0.17	
	A22 210'	410	2.49	0.17	0.91	0.16	<b>n = 6883</b>  <b>Mean Length = 2.72 <math>\mu\text{m}</math></b>  <b>Mean Length StD = 0.35 <math>\mu\text{m}</math></b>  <b>Mean Width = 0.82 <math>\mu\text{m}</math></b>  <b>Mean Width StD = 0.15 <math>\mu\text{m}</math></b>
		725	2.47	0.20	0.84	0.16	
		1189	2.79	0.29	0.77	0.09	
		476	2.44	0.18	0.78	0.11	
		419	2.29	0.22	0.89	0.13	
		642	3.11	0.25	0.85	0.15	
		1508	2.79	0.25	0.76	0.12	
		568	2.48	0.18	0.88	0.14	
		418	3.17	0.25	0.88	0.19	
		528	2.85	0.31	0.91	0.15	

	A22 480'	1499	3.09	0.21	0.93	0.20	<b>n = 3023</b>
		916	2.68	0.20	1.08	0.19	<b>Mean Length = 2.97 <math>\mu\text{m}</math></b>
		608	3.11	0.19	0.99	0.20	<b>Mean Length StD = 0.28 <math>\mu\text{m}</math></b>
							<b>Mean Width = 0.99 <math>\mu\text{m}</math></b> <b>Mean Width StD = 0.21 <math>\mu\text{m}</math></b>
Ca. T. hyper	Control	408	3.86	0.30	0.86	0.18	<b>n = 1634</b>
		426	3.56	0.39	0.89	0.17	<b>Mean Length = 3.65 <math>\mu\text{m}</math></b>
		219	3.17	0.23	0.90	0.18	<b>Mean Length StD = 0.43 <math>\mu\text{m}</math></b>
		365	3.50	0.24	0.90	0.20	<b>Mean Width = 0.90 <math>\mu\text{m}</math></b>
		216	4.21	0.34	1.03	0.23	<b>Mean Width StD = 0.20 <math>\mu\text{m}</math></b>
	A22 210'	281	3.66	0.25	1.01	0.15	<b>n = 1937</b>
		398	3.54	0.29	1.09	0.24	<b>Mean Length = 3.63 <math>\mu\text{m}</math></b>
		449	3.40	0.26	0.95	0.17	<b>Mean Length StD = 0.36 <math>\mu\text{m}</math></b>
		399	3.64	0.26	0.97	0.20	<b>Mean Width = 0.98 <math>\mu\text{m}</math></b>
		410	3.95	0.42	0.91	0.16	<b>Mean Width StD = 0.20 <math>\mu\text{m}</math></b>
	A22 480'	466	3.90	0.33	1.06	0.20	<b>n total = 1978</b>
		786	3.78	0.33	0.91	0.18	<b>Mean Length = 3.80 <math>\mu\text{m}</math></b>
		726	3.77	0.36	0.99	0.20	<b>Mean Length StD = 0.34 <math>\mu\text{m}</math></b>
							<b>Mean Width = 0.98 <math>\mu\text{m}</math></b> <b>Mean Width StD = 0.20 <math>\mu\text{m}</math></b>

**Table S2. Morphometric analysis of untreated and A22-treated *Ca. T. oneisti* and *T. hypermnestrae*, Related to Figure 6. StD: standard deviation.**

Species	Treatment	nc (n)	nc (%)	c (n)	c (%)	Total
<i>Ca. T. one</i>	Control	258	78.90	69	21.10	<b>(n) nc = 5963</b> <b>(n) c = 1963</b> <b>(%) nc = 75.12</b> <b>(%) c = 24.88</b>
		433	75.57	140	24.43	
		551	79.51	142	20.49	
		307	72.24	118	27.76	
		625	75.21	206	24.79	
		1312	77.31	385	22.69	
		838	73.00	310	27.00	
		719	73.97	253	26.03	
		573	73.94	202	26.06	
		347	71.55	138	28.45	
	A22	527	82.09	115	17.91	<b>(n) nc = 7093</b> <b>(n) c = 970</b> <b>(%) nc = 85.65</b> <b>(%) c = 14.35</b>
		1370	90.85	138	9.15	
		471	82.92	97	17.08	
		319	76.32	99	23.68	
		416	78.79	112	21.21	
		1470	95.70	66	4.30	
		846	85.20	147	14.80	
		788	95.86	34	4.14	

		482	89.59	56	10.41	
		404	79.22	106	20.78	
Ca. T. hyper	Control	284	69.61	124	30.39	(n) nc = 1143
		318	74.65	108	25.35	(n) c = 491
		162	73.97	57	26.03	(%) nc = 69.95
		221	60.55	144	39.45	(%) c = 30.05
		158	73.15	58	26.85	
	A22	254	90.39	27	9.61	(n) nc = 1631
		313	78.64	85	21.36	(n) c = 306
		369	82.18	80	17.82	(%) nc = 84.20
		332	83.21	67	16.79	(%) c = 15.80
		363	88.54	47	11.46	

**Table S3. Percentage of constricted and non-constricted cells in untreated and A22-treated *Ca. T. oneisti* and *T. hypermnestrae*, Related to Figure 6.** nc: non-constricted; c: constricted.



Species	Treatment	n	Kolmogorov-Smirnov p-value*	Shapiro-Wilk p-value*	Mean	StD	Std. Error Mean	t-Test p-value <sup>1</sup>
Ca. T. one	Control	10	0.200	0.570	24.880	2.725	0.862	<b>0.001</b>
	A22	10	0.200	0.419	14.346	7.021	2.220	
Ca. T. hyper	Control	5	0.200	0.079	29.614	5.831	2.608	<b>0.003</b>
	A22	5	0.200	0.705	15.408	4.805	2.149	

**Table S4. Tests for normal distribution and t-Test for % of constricting untreated and A22-treated *Ca. T. oneisti* and *T. hypermnestrae*, Related to Figure 6. (\*)  $p > 0.05$ , samples follow a normal distribution; (<sup>1</sup>)  $p < 0.05$ , controls and A22 are significantly different; StD: standard deviation.**

## SUPPLEMENTAL REFERENCES

- S1. Ouzounov, N., Nguyen, J.P., Bratton, B.P., Jacobowitz, D., Gitai, Z., and Shaevitz, J.W. (2016). MreB orientation correlates with cell diameter in *Escherichia coli*. *Biophys. J.* 111.
- S2. Fenton, A.K., and Gerdes, K. (2013). Direct interaction of FtsZ and MreB is required for septum synthesis and cell division in *Escherichia coli*. *EMBO J.* 32, 1953–1965.